MODIFICATION OF THE RATE OF OXYGEN CONSUMPTION BY CHANGES IN OXYGEN CONCENTRATION IN SOLUTIONS OF DIFFERENT OSMOTIC PRESSURE

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INTRODUCTION

It has not been possible to derive a general rule describing the relation between rate of oxidations in organisms and the oxygen tension of the environment. Pflüger (1872), reasoning from observations on the oxygen-absorbing power of the blood and the partial pressure of oxygen in the alveoli of the lungs in the dog, concluded that the rate of oxygen absorption of the tissues is independent of the oxygen tension over a wide range. Thunberg (1905), testing the oxygen absorption of *Lumbricus*, *Limax*, and *Tenebrio* larvæ in different oxygen tensions, found that the rate varies with the oxygen tension. Thunberg assumed that the oxygen tension within the cells is less than that of the milieu, but not zero, and concluded that his results were in accord with the fact that the velocity of a reaction depends on the number of molecules reacting; hence the velocity of oxidations in the organism varies with the oxygen concentration of the environment until a maximum is reached. Henze (1910), from his experiments with pelagic forms, concluded that in organisms with thin and delicate tissues the oxygen tension of the cells is that of the surrounding water and always in excess of requirements; consequently in such organisms the rate of oxygen utilization is independent of the oxygen tension of the water.

Krogh (1916) amplified Henze's conclusion and states that whether or not the rate of oxygen consumption is independent of the oxygen concentration of the environment depends on two conditions: In animals with well developed blood streams and respiratory systems, and in animals with a low percentage of dry substance, the oxygen tension does not affect the rate of oxygen utilization over a wide range. The assumption is necessary in these cases that the oxygen tension in the tissues is positive. On the other hand, if the organism has a high proportion of dry substance and "imperfect" respiratory and circulatory systems, the rate of oxygen utilization is highly

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dependent on the oxygen tension of the environment until the oxygen tension is above the normal. It is assumed that the oxygen tension of the tissues of such forms approaches zero.

Hyman (1929) suggests that, in certain cases at least, the body surface acts as a regulatory mechanism; this appears to act according to internal requirements and hence in the presence of excess oxygen the oxidative rate remains constant. Shoup (1929) holds that the size of the organism is important. In general, according to Shoup, microscopic forms are small enough to allow complete diffusion of the dissolved gases. In other words, they are independent of the oxygen concentration, within limits, because oxygen is always present in excess within the system. Shoup also adopts the view expressed by Krogh (1916) that in large organisms the rate of respiratory exchange depends on oxygen tension and the adequacy of the respiratory and circulatory systems.

Thus far it is not possible to explain the experimental evidence at hand by any or all of these theories. According to Krogh, the water content of the organism is an important characteristic in determining its response to changes in the oxygen tension of the environment. Thus an organism with relatively low water content and what Krogh calls "imperfect" systems of respiration and circulation might be expected to consume oxygen in direct dependence on the oxygen concentration of the environment. In the case of *Planaria dorotocephala* certainly the mechanisms of respiration and circulation are, so far as known, simple and rudimentary; yet Hyman (1929) found that this form has a constant rate of oxygen utilization over a range of environmental oxygen tension between 3 cc. and 9 cc. per liter. If Krogh's suggestion has a basis in fact, one would expect that the water content of this animal is relatively high.

Planaria afford suitable material for a further study of the relation between water content and the degree of dependence of rate of oxygen consumption on the oxygen tension of the milieu. More specifically, the problem at hand is: What is the mechanism that renders the rate of oxygen utilization of this animal indifferent to changes in the oxygen tension of the surrounding medium? Krogh's suggestion indicates the point of attack, namely, the relation between water content and the effect of changes in oxygen tension on the rate of oxygen utilization with changes in free water content of the organism.

Methods [1]

Investigation of this problem resolved itself into (a) finding the total free water content of *Planaria dorotocephala;* (b) increasing and decreasing the water content by means of hypo- and hypertonic solutions; (c) determining the rate of oxygen consumption of the animals in different concentrations of oxygen in natural water, and in hypo- and hypertonic solutions.

The free water content was determined by weighing the animals after removal from water, then drying over sulphuric acid in a desiccator, and weighing repeatedly until no further decrease in weight occurred. The first weighing was accomplished as follows: The animals were poured in water into a properly folded hard-surfaced filter paper, the water drained off, and the paper drawn across a plate of glass until it adhered firmly. The animals were then picked up with a thin spatula and placed in a weighing tube, the tube closed, and the weighing completed as rapidly as possible. The proceeding was timed so that the exposure of the animals to air was approximately the same in different experiments. Weighing the animals while wet introduces the error of the weight of water adherent to their surfaces. Considerable success was realized in standardizing this error, for during exposure to sulphuric acid the animals lost very nearly the same percentage of total weight in all experiments; which indicates that the original proportion of adherent water must have been much the same in each first weighing. The total loss of weight as shown by the data must be discounted by the weight of adherent water at the first weighing. The actual water content of the animals is therefore somewhat less than the data indicate.

The degree of dryness attained by desiccation over sulphuric acid depends on the relative affinities of the acid and of the animal tissues for water. The method was used by Davenport (1899) in his analysis of the changes in water content of the frog embryo during development. For the present work the method is more satisfactory than heating to dryness, for only free water is withdrawn from the organism. Heating to dryness involves loss of bound water as well as other substances. It is not assumed, however, that drying over sulphuric acid actually removes all the free water from the system, for the resistance of aquatic organisms to desiccation is common. However, the proportion of water remaining after the treatment must be very small; the residue is a brown mass that on pressure breaks up into a coarse granular powder.

The hypo- and hypertonic solutions employed throughout this work were distilled water and Ringer's solution made up with buffer and in concentration that is described as isotonic for amphibian tissue.

The effect of distilled water on the weight of *Planaria dorotocephala* and the analysis of the distilled water used are given in a former paper (Buchanan, 1930a). It was also shown that these animals in some way condition distilled water; accordingly in this work the proportions of animal tissues to distilled water were made approximately the same in all experiments. Since the changes in weight in hypo- and hypertonic solutions constitute an important phase of the problem at hand, the method employed in obtaining the data given in the former paper is restated.

Twenty-five animals of medium length were weighed and placed in 250 cc. of distilled water, then weighed again after certain intervals. The hydrogen ion concentration of the distilled water was reduced by the addition of a minute amount of NaOH. The experiments were conducted at room temperature, 20° to 23°. Control lots were weighed after similar handling in tap water and the difference in changes in weight between the distilled and tap water lots is regarded as due to the effect of distilled water on weight. The uniformity of the results was seriously interfered with by the fact that the distilled water causes the animals to exude slime. It is difficult to remove this slime during the weighing process. Furthermore, partial drying on filter paper and the incidental exposure to air causes a withdrawal of water from animals that have taken up water from a hypotonic medium. Thus the data show wide variations in weight changes in distilled water. It is clear, however, that after four hours in distilled water, pH 6.8 to pH 7.6, the weight increases. Consequently for respiration tests I employed animals that had been pre-treated with distilled water for from four to six hours.

The effect of Ringer's solution on the weight of *Planaria dorotocephala* is also given in a former paper (Buchanan, 1930b). Ringer's solution was employed because in the writer's experience the animals live longer in this hypertonic medium than in others that have been tried. At 20° medium sized *Planaria* will live for several days in Ringer's solution. Data on the effect of Ringer's on the weight of the animals were obtained by methods similar to those employed for distilled water. The results are fairly uniform, for the animals do not exude slime. It is shown that the weight decreases continuously during the first four hours of exposure, but after that time further loss of weight is slight. Hence, for tests of the effect of loss of water from the animals on their rate of oxygen consumption under different

conditions of oxygen concentration, I employed animals that had been exposed for four hours previously to Ringer's solution.

The oxygen content of the various liquids was determined by the Winkler method. This method has recently been criticised by Shearer (1930). His criticisms are based in large part on his statement that when present in numbers a European planarian discharges substances which should, according to Shearer, absorb iodine and thus interfere with the titrations. Shearer assumes that this possibility has not occurred to us. The fact is rather elementary that in iodometric work the possible absorption of iodine by substances in the solution must be taken into account. Years ago Hyman (1919) showed conclusively that the substances which *Planaria dorotocephala* excretes do not absorb iodine. Further evidence is to be found here. *Planaria* exude slime in distilled water but none at all in Ringer's solution, yet the titrations show that the oxygen consumption of the animals is materially less under comparable conditions in distilled water than in Ringer's.

Shearer also criticises work done with *Planaria* by this method on the grounds that the motor activity of the animals is not controlled. Shearer himself attempts to draw conclusions concerning the motor activity of an American flatworm which he probably has never seen, from quite unconvincing evidence obtained from a European form. If the head of *Planaria dorotocephala* is removed the animal continues to crawl about for some minutes. Then it comes to rest in a contracted state and if undisturbed does not change position for many hours. This is also true of *Planaria maculata* but not true of *Phagocata gracilis*. A comparison of the rate of oxygen consumption of beheaded *Planaria dorotocephala* with that of intact animals brought to rest by other methods, namely, lowering the temperature slightly and excluding light and other stimuli, shows no appreciable difference (Hyman, 1919*a*, 1919*b*; Buchanan, 1926). It has thus far been impossible in this, as in much of the work on standard metabolism, to exclude local muscular twitching and the action of cilia, without recourse to profound anesthesia. If the work on *Planaria* is invalidated by these internal and localized motor activities, then the same standards must be applied to a very considerable proportion of the work on respiratory metabolism, including Shearer's. In the present work the factor of motor activity of the animals is not seriously concerned, for the respiratory differences are of such nature and so conspicuously different that they cannot be explained on the basis of differences in motor activity.

The possibility of the salts in Ringer's interfering with the titrations

required safeguarding. Since only the relative quantities of oxygen in the medium before and after the animals had consumed some of it were of major importance, the action of salts may be discounted so long as it permits a sharp end point. To assure the accuracy of titration, a casserole containing the same quantity of Ringer's as the sample to be titrated was placed beside the casserole containing the sample. To this Ringer's was added sufficient iodine to match the color of the sample. It was then cleared with sodium thiosulphate. This served as a check end point, and the sample was then cleared to the same state of decoloration as the check. The end point in Ringer's was quite distinct and it was found unnecessary to provide this check as a constant procedure.

A much more difficult problem was the control of motor activity so that standard conditions obtained in the several environments. In part this was solved by the fact that *Planaria dorotocephala*, when changed from light to darkness, or when changed to a somewhat lower temperature, remain quiet for some hours. In this work both of these methods were employed. In both tap and distilled water the factor of motor activity does not enter, for in both the animals were quiet throughout the tests, except for the very brief time at the start, when the animals were distributing themselves about the interior of the flasks. In Ringer's solution the animals writhe for some time, then become paralyzed, accumulating at the bottom of the flask in abnormal positions. After two hours or less only occasional spastic movements occur unless light is admitted or the flask agitated. Unquestionably such twitching results in the consumption of oxygen. If one may judge from observations on the extent of such localized muscular activity, the amount of oxygen required for this movement must be very small as compared with the total oxygen consumption. Furthermore, there is no evidence in the observations that the extent of such movements increases with the increase in oxygen concentration of the Ringer's. After careful observation of the behavior of the animals as compared with the oxygen consumption under the several conditions, one must conclude that the differences in oxygen consumption cannot be ascribed to differences in motor activity.

In carrying out a titration the quantity of clearing agent used in reaching the end point is in practice slightly in excess or else slightly under the exact quantity. Also in the necessary manipulations no two lots of animals can be treated in exactly the same fashion. In order to compensate for such variations in procedure that result in a spread in the results, duplicate lots of animals were carried simultaneously in all experiments and the results recorded are the average

between them. The oxygen consumption of such duplicates varied in amount, sometimes rather widely. But with one or two exceptions both members were in the same direction from the control determinations and fell within ranges that are consistent with the general data.

If the oxygen concentration of the medium is reduced materially below saturation at room temperature, some oxygen will be absorbed during a two-hour experiment, from the air or from the wall of the flask, or by leakage through the connections. Likewise, if the oxygen concentration is higher than saturation at room temperature, oxygen will be lost during the experiment. Obviously, therefore, determinations of the oxygen content of flasks in which animals had been kept for two hours had to be corrected for changes in oxygen concentration not due to the metabolism of the animals. A considerable series of tests was made in an attempt to determine the magnitude of change at various oxygen concentrations. The apparent oxygen consumption of the animals was then corrected in each case in accordance with the average oxygen gain or loss at approximately the same oxygen tension in tap H_2O in the absence of animals. Admittedly this does not yield an absolutely accurate result, for the control tests just described showed that the amount of oxygen gain or loss is subject to some variation. However, this appears to be the most accurate and convenient method at hand for establishing the necessary correction. A tabulation of the average oxygen gain or loss at various oxygen tensions which was used as a table of corrections is given as Table III.

A typical experiment to test the effect of differences in osmotic pressure of the medium on the response of the organism to different oxygen concentrations was carried out as follows: Twenty-four hours before the test, if distilled water was to be used, a large quantity of distilled water was treated with carbon dioxide-free air, the water being treated for at least twelve hours. The H ion concentration was thus decreased to pH 7.0 prior to use in the experiment. A selected lot of animals was divided into two groups and their rates of oxygen consumption in tap water were taken over a two-hour period. In all cases the oxygen concentration of the tap water was at or near saturation at 20°; the H ion concentration being always lower than pH 7.0 but never as low as pH 7.8. The amount of oxygen consumed per two hours under these conditions is regarded as the normal and in comparison with the effects of other conditions is taken as 100 per cent. The animals were then placed in 500 cc. of distilled water, or of Ringer's, as the nature of the experiment required, the oxygen tension of which was approximately that of the tap water in the control test. After four or more hours a fresh supply with higher or lower oxygen

tension, but with the same H ion concentration, was siphoned into the flasks, after appropriate washings, which were then sealed and immersed in the constant temperature bath in darkness. Samples for titration were taken of the stock solutions and of the contents of the flasks at the end of two hours.

The oxygen concentration of the stock solutions was controlled either by bubbling pure oxygen through the stock, or by driving out most of the oxygen by heating and then cooling in a container so sealed as to prevent the ingress of air directly to the supply to be used. The time intervals were accurate to within one minute, the temperature within 0.5°, and the H ion concentration ranged in the various experiments from pH 7.0 to pH 7.8. In the case of Ringer's the pre-treatment with carbon dioxide-free air was not necessary, since the solution was buffered to pH 7.8. In all cases the test containers were Erlenmeyer flasks, capacity 500 cc., in which were placed from 35 to 60 animals, depending on size. Over a two-hour period the products of the animals increased the H ion concentration of distilled water approximately pH 0.2, somewhat less in tap water, and not at all in Ringer's. Therefore at no time was the H ion concentration greater than pH 6.8, nor less than pH 7.8. According to Hyman (1925), within this range the effect of H ion concentration on oxygen consumption is slight.

<center>DATA</center>

Table I shows that the water content of *Planaria dorotocephala* is approximately 78 per cent of the total weight, as determined by drying over sulphuric acid. The accuracy of this value is of course conditioned by the weight of water adherent on the surfaces of the animals at the first weighing and by the distribution of water between the drying agent and the organic material. The affinity of the latter for water being undetermined, it is not justified to assume that all the free water is removed by this method. With these reservations, it is, however, certain that the water content of these animals is relatively low. Comparison of these data with those given by Davenport (1896, pp. 58–59) for other invertebrates shows that the water content of *Planaria* is comparable with that of other soft forms, although somewhat lower. It is much lower than that of hyaline forms referred to by Krogh (1916, pp. 58–59). Thus the observed facts do not support Krogh's hypothesis, that independence of rate of oxygen utilization of environmental oxygen supply is conditioned on a low content of dry substance.

The effect of distilled water and of Ringer's solution on the weight of *Planaria* is given in two former papers (Buchanan, 1930a, 1930b).

Table I

Loss of weight of Planaria dorotocephala over sulphuric acid. All animals starved two weeks before weighing. In milligrams and percentage change.

Length	No. of Animals	Live Weight	Final Weight	Loss
mm.		*mg.*	*mg.*	*per cent*
15	5	46.2	9.8	78
"	8	61.9	14.1	77
"	11	74.6	20.0	73
"	14	109.1	23.7	78
"	18	131.2	28.1	78
"	25	180.5	38.6	78
"	20	142.4	34.0	75
"	20	134.4	31.7	76
"	20	143.7	28.2	80
"	20 +	160.5	34.4	78
16	20	196.7	36.7	81
"	20	194.7	39.2	79
"	20 −	159.7	36.3	77
"	20 +	223.3	44.7	79

Table II is abstracted from the data there given and consists of weight changes in experiments in which the conditions were comparable to those which were employed in testing the effect of these environments on respiration. As stated above (p. 312), it was found impossible to weigh animals from distilled water with accuracy, due to the adherent slime and the accidental drying that occurs when the animals are handled on filter paper. The average weight gain shown by the data is approximately 15 per cent; the organism being 78 per cent water, this represents an increase in total free water of 11 per cent. No great confidence may be placed on these data, however. At best they merely indicate that water is taken up by the animal while in distilled water.

Table II

Changes in weight of Planaria dorotocephala after four- and five-hour treatment with Ringer's solution and distilled water. Abstracted from Table III, 1930a, and Table III, 1930b. All animals starved at least one week. 14–18 mm. animals. Twenty-five animals in 250 cc. of liquid in each case. Room temperature, Ringer's solution, pH 7.8; distilled water, pH 7.0 to pH 7.6. In percentage change as compared with controls in tap water.

Ringer's Solution		Distilled Water	
4 hours	5 hours	4 hours	5 hours
− 17.7	− 17.7	+ 1.9	+ 24.6
− 17.7	− 19.0	+ 3.7	+ 18.6
− 20.9	− 18.5	+ 4.5	+ 16.5
− 16.9	− 14.2	+ 1.9	+ 11.9
− 17.0		+ 0.6	− 2.8
− 10.0		+ 1.5	− 1.1
− 11.0			
− 12.0			
Average − 16%		+ 15%	

In the case of Ringer's, the data show clearly that the water loss amounts to approximately 16 per cent of the total weight, or a reduction of approximately 20 per cent of the total water content.

Figure 1 shows in graphic form the effects of changes in the oxygen tension of the medium on the rate of oxygen consumption in distilled
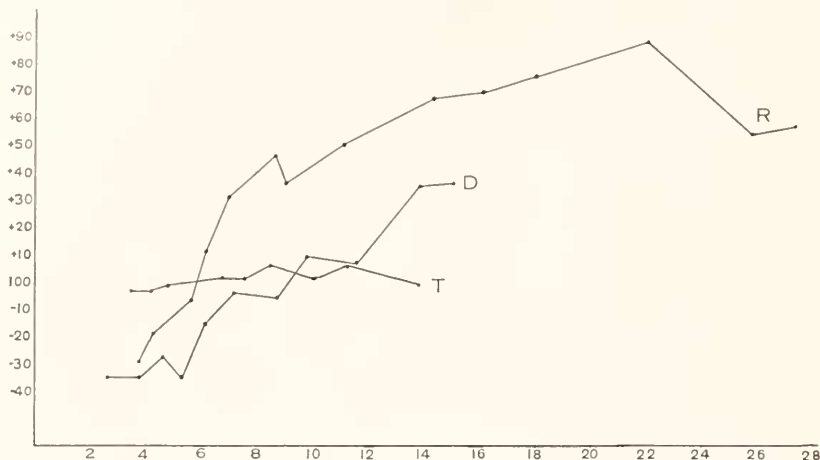

FIG. 1.   The effect of increase in oxygen tension of the medium on the oxygen consumption of *Planaria dorotocephala*.   Abscissæ represent cc. oxygen per liter. Ordinates represent changes in rate of oxygen consumption as compared with rate in tap water with oxygen concentration at or near saturation at 20°, which is taken as 100 per cent.   T, animals in tap water; D, animals in distilled water; R, animals in Ringer's solution.   Temperature, 20° ± 0.5°.   H ion concentration, pH 6.8 to pH 7.8.

water, tap water, and Ringer's solution.   The points on the respective graphs were determined by consolidating the data as follows: For each oxygen concentration duplicate lots of animals were used and the average between them is set down as the result at that particular oxygen concentration.   Up to 8 cc. of oxygen per liter the determinations within a range of 1 cc. of oxygen per liter were averaged and plotted.   Above 8 cc. per liter determinations of oxygen consumption within each concentration range of 2 cc. per liter were averaged and plotted.   The graphs, then, represent 52 separate determinations of oxygen consumption in distilled water, 54 determinations in tap water, and 68 determinations in Ringer's solution.

The graph of the controls in tap water, C in Fig. 1, shows that within the range of oxygen tensions employed the rate of oxygen consumption does not change materially.   For a number of years, in connection with other problems, data have accumulated on the rate

TABLE III

Average change in oxygen concentration in sealed 500 cc. flasks of tap water immersed in a water bath at 20° for two hours. Corrections for the data plotted in Fig. 1 were taken from this table. Change in percentage.

Range of Oxygen Concentration	No. of Experiments	Average Change
cc./liter		*per cent*
20–30	12	− 0.9
15–19	8	− 0.6
10–14	16	− 0.5
7–9	24	− 0.2
6	4	0.0
5	8	+ 0.1
4	4	+ 0.2
3	12	+ 0.6

of oxygen consumption of *Planaria dorotocephala* in oxygen tensions varying around the saturation point of water at room temperature. The data are in terms of the oxygen consumed per unit of wet weight per unit of time. At hand are 282 determinations that were made under comparable conditions; temperatures were between 18° and 24°; 208 experiments were conducted at 20°. The calculations necessary to determine the relationship between rate of oxygen utilization and the oxygen tension of the water have been carried out.[2] The results are as follows:

Number of experiments, 282.
Highest temperature of an experiment, 24° (3 cases).
Lowest temperature of an experiment, 18° (9 cases).
Average temperature, 20°.
Oxygen concentration of the water:
       Extremes, 3.90 and 7.19 cc. per liter.
       Mean, 5.87 cc. per liter.
       Standard deviation, 0.64 ± 0.018.
Oxygen consumption, per gram per two hours:
       Extremes, 0.30 cc. and 0.64 cc.
       Mean, 0.37 cc.
       Standard deviation, 0.05 ± 0.0014.
Coefficient of correlation, oxygen consumption and oxygen tension, 0.09 ± 0.04.

While a statistical treatment of data of this sort is not free from criticism, the fact that the coefficient of correlation is not significant, when considered together with the graphic results shown in Fig. 1,

[2] These calculations were made by Miss Rosalthea Sanders.

and the data of Hyman (1929), constitute convincing evidence that the rate of oxygen consumption of *Planaria* in natural water is independent of the oxygen tension over a wide range.

Figure 1 also shows that in distilled water after four to six hours the rate of oxygen consumption is sub-normal and remains fairly constant between oxygen concentrations of 2.65 and 5.35 cc. per liter.[3]
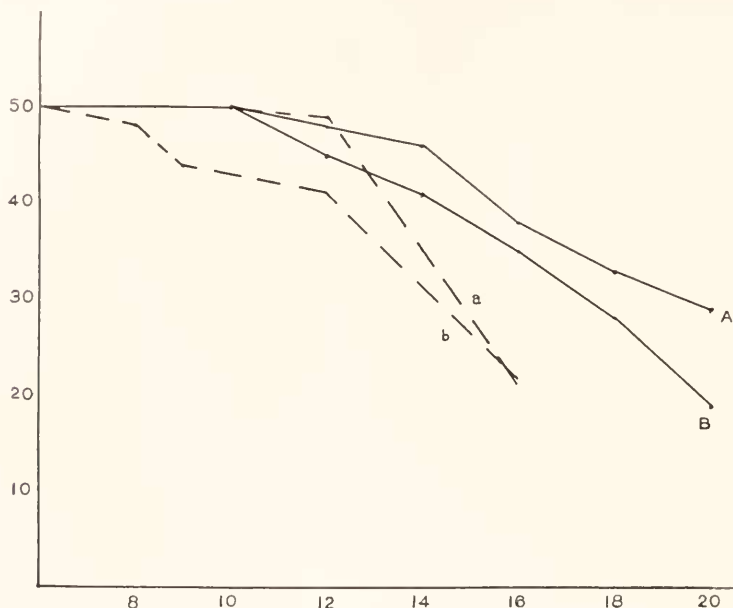

FIG. 2.   Rate of disintegration of *Planaria dorotocephala* in distilled water with different oxygen content.   Ten animals in 500 cc. distilled water in each case.   Room temperature.   Abscissæ represent hours; ordinates are derived from arbitrary values assigned to portion of animals alive at each observation, *e.g.*, 50 indicates that all 10 within a flask are alive; 40 indicates that some have partially disintegrated. *A* and *B* and *a* and *b* represent two different experiments.   In *A* the oxygen concentration of the distilled water was 11.94 cc. per liter; in *B* the oxygen concentration was 6.16 cc. per liter.   In *a* the oxygen concentration was 17.75 cc. per liter; in *b* the oxygen concentration was 6.51 cc. per liter.

It increases as the oxygen concentration is further increased, but is below that in tap water until the oxygen tension is increased beyond

[3] There is an apparent discrepancy between these results for distilled water and those of Hess (1929) and those given in an earlier paper by the writer (Buchanan, 1929*a*).   The depressive action of distilled water shown here arises from the fact that the animals were pre-exposed, after washing with distilled water, from four to six hours, then washed by siphoning distilled water through the flasks for several minutes, then sealed in distilled water for two hours, thus bringing the total exposure to distilled water to totals of six and eight hours, in large volumes.   The earlier results (1929*a*) and those of Hess (1929) agree that long exposure to distilled water lowers the rate of oxygen utilization.   The data here given are not in conflict with those results.

9 cc. per liter. In Ringer's solution the fact appears that the rate of oxygen consumption is sharply dependent on the oxygen tension of the solution until the tension reaches a high value, approximately 14 cc. per liter. The effect of increasing the oxygen concentration above 14 cc. per liter becomes increasingly less; the data indicate that in the highest concentrations employed the rate of oxygen consumption is somewhat less than in lower concentrations.

Thus it appears that although the animals in natural waters are independent over a wide range of oxygen concentration, this is not a fixed condition, but is susceptible of being materially affected by either the salt content of the water, or by the osmotic pressure, or by both.

Figure 2 shows that animals in distilled water with a high oxygen content disintegrate slightly less rapidly than do animals in distilled water with oxygen content around the saturation point at room temperature. The method of plotting these data has frequently been described in detail and will not be re-described here. Reference to Fig. 1 shows that the rate of oxygen consumption of animals in distilled water containing corresponding concentrations of oxygen is higher in the higher concentrations, the reverse of the order of disintegration. In other words, in high oxygen concentrations the rate of oxygen utilization is greater while the rate of disintegration is lower than in normal oxygen tensions.

DISCUSSION

With regard to the original problem, what is the mechanism that renders the rate of oxygen consumption of this animal indifferent to oxygen concentration changes in the environment, these results render certain hypotheses improbable.

In the first place, it is clearly shown that this characteristic, in this particular animal at least, may be modified by the action of salts and by the osmotic pressure of the environment. As a matter of methodology, this fact is of considerable importance. For if one were to measure the oxygen consumption of an animal in Ringer's solution, for instance, with the oxygen tension slightly greater than saturation at 20°, the data would appear to show that Ringer's accelerates oxygen utilization. On the other hand, should the oxygen concentration of the Ringer's be somewhat under saturation at 20°, the results would indicate that Ringer's is a depressant. The effects of various salts on respiratory metabolism have received considerable attention, but there is little uniformity in the findings of various investigators. A partial review of the literature is given by Hess (1929).

Secondly, the results are important in showing that the absolute size of the animals, *i.e.*, the surface-volume relation, as suggested by Shoup (1929), may be rejected as a general factor in determining the relation between rate of oxygen utilization and oxygen tension. Also the rôle played by the degree of perfection of the respiratory and circulatory mechanisms does not appear to be of general importance; it is inconceivable that Ringer's or distilled water in any way converts these mechanisms in *Planaria*.

With these hypotheses of limited application, attention must be focused on the cellular oxidative mechanisms, not sufficiently emphasized in Krogh's (1916) discussion. The possibility must be considered that the relation between rate of oxygen utilization and the oxygen concentration of the environment is conditioned on the concentration of oxidative enzymes in relation to the oxygen concentration within the tissues. In the total absence of any information whatever as to the oxygen tension in the tissues, the permeability of the tissues to oxygen, and the effects of Ringer's solution and distilled water on tissue permeability to oxygen, explanations of the causal factors in these results must be in large part conjecture. However, sufficient facts are shown by the data to justify a discussion of certain possibilities.

In the normal animal in tap water, assuming that oxygen is present and oxidizable substances are in excess in the tissues, the velocity of oxidations is a product of a constant and the concentration of the active enzymes. That is,

$$Velocity = kE.$$

So long as E remains constant, further increase in the oxygen tension should have no effect on the rate of oxygen utilization. This constitutes a possible explanation of the fact shown in Fig. 1, that increasing the oxygen concentration of the tap water has no effect on the rate of oxygen utilization within the concentration range employed.

But if the value of E is increased, thereby increasing the value of V, the oxygen concentration in the tissues becomes a limiting factor until it reaches a value equal to the oxygen-transferring power of the enzymes as conditioned by the rapidity of adsorption of the substrates. If the value of E is decreased, V is proportionately decreased and remains constant so long as the oxygen tension is adequate to maintain a constant adsorption rate.

The data on both Ringer's and distilled water offer some support for this interpretation, for it will be noted in Fig. 1 that when the oxygen concentration reaches a certain value the rate of oxygen

consumption tends to become constant. In distilled water this value is low, between 2.65 cc. and 5.35 cc. per liter, as would be expected if the cell enzymes were diluted. It is unfortunate that exact data on the water increase in distilled water have not been determined with accuracy; a dilution of 11 per cent could hardly account for the lowered rate of oxygen utilization as compared with the rate in tap water. Still, there is no general law stating the relation between enzyme concentration and the rapidity of an enzyme reaction except that small amounts of an enzyme are relatively more effective than are greater concentrations. The data here are in accord with this, but their nature does not permit of further analysis in this direction.

The evidence is considerably more impressive in the data on the effects of Ringer's solution. The rate of oxygen consumption tends to become constant at approximately 14 cc. oxygen per liter, a result to be expected if the cell contents are materially concentrated. Reduction of water content in Ringer's is approximately 20 per cent. These suggestions regarding the effects of both distilled water and Ringer's solution on the oxygen utilization necessarily include the assumptions that the oxygen tension in the tissues is less than that of the surrounding medium and increases with increase in environmental oxygen concentration.

Under normal conditions, with environmental oxygen in excess of requirements, undoubtedly one of the conditions which determine the rapidity of oxygen utilization in the organism is the concentration of the oxidative enzymes. It is not unreasonable to suppose, and the results here support the view, that at least one of the conditions that determine the degree of dependence of oxidative rate on oxygen tension of the milieu is the relative concentration of the oxidative enzymes.

Another interpretation of lines T and R in Fig. 1 is possible. For this, two assumptions are necessary: (a) that in tap water with oxygen concentration between the minimum and maximum employed, the intra-cellular oxygen tension is greatly in excess of requirements, and (b) that Ringer's materially reduces permeability to oxygen, thus reducing intra-cellular oxygen tension. Under such conditions the rate of enzyme-controlled oxidations within the cell in tap water may be expected to remain constant with increase in environmental oxygen supply, while in Ringer's increasing the environmental oxygen concentration would be expected to effect corresponding increase in intra-cellular oxygen tension, with consequent increase in rate of oxygen utilization. The fact that in Ringer's the effects of increasing oxygen concentration form a logarithmic curve rising well above the

rate in tap water must be referred to the effect of Ringer's in extracting water and by concentration increasing the rate of oxygen transfer by the enzymes.

However, the fact that the effects of increasing the oxygen tension in distilled water above 5.35 cc. per liter are in general parallel to the effects in Ringer's, in that the rate of oxygen consumption is no longer independent of the supply, seems to indicate that some condition common to both distilled water and Ringer's disturbs the normal oxidative mechanism. Apparently, the ability of the oxidative enzymes to transfer oxygen is not adversely affected, for in both the rate of oxygen utilization increases above the controls with increase in oxygen concentration. Since in time both distilled water and Ringer's are lethal, it is conceivable that this common effect is an indication of the onset of structural breakdown. The most probable early injurious effect that would bring about this common effect is interference with the normal cell permeability to oxygen. While permeability effects are usually referred to the cell boundary and there is substantial evidence that the cell boundary does regulate the admission of oxygen (Warburg, 1911), permeability of the general cytoplasm must also be considered.

An interesting fact appears in the data on the effect of increasing oxygen tension in Ringer's solution. When the oxygen concentration of the Ringer's is approximately at saturation for that concentration of salts at 20°, the rate of oxygen consumption is approximately that of normal animals in natural water. Oxygen being less soluble in Ringer's than in water at the same temperature, this saturation point therefore represents an environmental oxygen supply somewhat less than at saturation in tap water. The significance of this fact is not apparent; it may be merely incidental with this particular concentration of salts.

Figure 2 shows that oxygen in the concentrations employed is not toxic for *Planaria*. The animals disintegrate slightly less rapidly in abnormally high concentrations of oxygen in distilled water than in distilled water with oxygen tension around the saturation point at room temperature. Also, it may be pointed out, with reference to Fig. 1, that in distilled water at oxygen concentrations corresponding to those used in Fig. 2 the rate of oxygen consumption is higher in the higher concentrations, while Fig. 2 shows that the rate of disintegration is slightly lower in the higher oxygen concentrations. In other words, the higher the concentration of oxygen the less rapid the rate of disintegration and the higher the oxygen consumption. These facts substantiate the conclusion expressed in a former paper (Buchanan,

1930*a*), that disintegration in distilled water is not a direct result of the action of the hypotonic medium on the oxidative metabolism of the animal; that relative rate of disintegration in distilled water is not an index of relative rate of oxidative metabolism.

Summary

In tap water the rate of oxygen consumption of *Planaria doroto-cephala* is independent of the oxygen concentration of the water between 3.53 cc. and 13.84 cc. per liter. In so far as the methods coincide, this confirms the results of Hyman (1929).

In Ringer's solution after four hours' exposure *Planaria* lose approximately 16 per cent of their total weight, or approximately 20 per cent of their total water content. In Ringer's the rate of oxygen consumption is sharply dependent on the oxygen concentration of the solution between 3.79 cc. and approximately 14 cc. per liter. Above this concentration the rate of oxygen consumption tends to become constant.

In distilled water the animals imbibe water; irregular data indicate that during the first four or five hours of exposure the weight increases on the average 15 per cent, which represents an increase in water content of approximately 11 per cent. The rate of oxygen consumption in distilled water is lower than normal and constant in oxygen concentrations between 2.65 cc. and 5.35 cc. per liter. Above this concentration the rate of oxygen consumption increases as the oxygen tension increases, rising above the normal at approximately 9 cc. per liter, and continues to increase as the oxygen concentration increases, up to 15 cc. per liter, the maximum employed.

Planaria dorotocephala contain approximately 78 per cent free water, as shown by weight loss on desiccation over sulphuric acid.

The facts indicate that two conditions may be involved in the regulation of the rate of oxygen utilization in relation to oxygen tension in natural water: Concentration of water within the organism, thus controlling the concentration of the oxidative enzymes; boundary regulation of the admission of oxygen. In this animal, size (surface-volume) and the degree of development of its circulatory and respiratory mechanisms do not appear to be important.

In abnormally high oxygen tensions in distilled water the rate of oxygen consumption is higher and the rate of disintegration is lower than in distilled water with oxygen tension at saturation at 20°.

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